CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- DNOC is rapidly absorbed following inhalation, oral, or dermal exposure.
- Limited human and animal data indicate that absorbed DNOC is distributed to most tissues, but does not accumulate in any particular tissue.
- Available animal data indicate that DNOC is biotransformed to less toxic metabolites; conjugation represents only a minor pathway to elimination.
- DNOC appears to be metabolized to less toxic metabolites readily eliminated via the urine. Although small quantities of DNOC may be conjugated, most of the dose appears to be reduced to mono amino derivatives and then subsequently conjugated prior to excretion. These relatively harmless metabolites have been found in the urine and kidney of humans and animals exposed to DNOC.

3.1.1 Absorption

DNOC is rapidly absorbed by the respiratory tract in humans and animals. A serum DNOC concentration of 1,000 µg/mL was detected in a spray operator 24–36 hours after inhaling a dense DNOC mist for an acute duration (van Noort et al. 1960). A blood DNOC concentration of 60 µg/g was detected in a spray operator who had been exposed to DNOC during mixing and/or spraying operations over a 5-week period (Pollard and Filbee 1951). In an occupational exposure study involving DNOC manufacturers, winter-washer sprayers, and cereal-crop sprayers, a correlation between blood DNOC levels and the symptoms and signs of poisoning was observed (Bidstrup et al. 1952). These data indicate that DNOC was absorbed from the respiratory tract; however, dermal absorption may have been involved as well. Limited studies in rats also show that DNOC is absorbed after inhalation exposure (King and Harvey 1953a, 1954).

DNOC is readily absorbed by the gastrointestinal tract in humans and animals. The detection of DNOC in liver, stomach, kidney, heart, and brain of two humans who committed suicide by ingesting DNOC provides evidence of gastrointestinal absorption (Sovljanski et al. 1971). DNOC was readily absorbed when 75 mg DNOC/day was given to five volunteers for 5 days (Harvey et al. 1951; King and Harvey 1953b). Further analysis of the data for these volunteers revealed that they excreted approximately 7% of the total DNOC dose in the urine over 13 days from the first dosing days (King and Harvey 1953b). In

the first 24 hours after a single dose of 75 mg, an average of 39.2% of the dose could be accounted for by blood levels and 1.3% by urinary levels.

Studies in animals reveal differences among species. Maximum blood DNOC concentrations of 72.2 µg/g at 6 hours after the last dose of 20 mg/kg/day for 9 days and 105 µg/g at 3.5 hours after a single dose of 30 mg/kg DNOC were found in rats (King and Harvey 1953b). The gastrointestinal absorption of DNOC was approximately 20, 10, and 5% of the dose at 1, 2, and 7 hours after dosing, respectively. When rabbits were similarly treated, peak values were 54.7 µg/g at 4.5 hours after multiple doses of 25 mg/kg/day DNOC and 49.5 µg/g at 6 hours after a single dose of 30 mg/kg. Blood DNOC levels of 25, 34, and 50 µg/g were detected in rabbits given single oral doses of 10, 15, or 18 mg/kg DNOC, respectively (Truhaut and De Lavaur 1967).

In two rats given oral doses of 0.4 mg/kg ^{14}C-DNOC, 60% of the radioactive dose was accounted for in blood, urine, and tissues from one rat that was killed 1 day later and the other rat that was killed 3 days later (Leegwater et al. 1982). In the rat killed 1 day later, 15% of the radioactive dose was detected in the blood, while 28.7% was accounted for in the urine and approximately 41% was distributed to other body tissues. In the rat killed 3 days after dosing, 5.5% of the radioactive dose was detected in the blood, while 41% was accounted for in the urine and approximately 20% was distributed to other body organs.

As part of a study to determine the influence of dietary fats on the absorption of DNOC, mean blood levels of 50.7, 71.0, 81.0, 76.3, 61.4, 42.6, 28.3, and 19.1 µg/mL DNOC were detected at 15 minutes, 1, 3, 6, 12, 24, 30, and 48 hours, respectively, after rats were given a single dose of 15 mg/kg DNOC in saline (Starek and Lepiarz 1974). Gavage administration of olive oil, rape seed oil, or castor oil immediately after DNOC resulted in some alteration of these blood levels, indicating that the influence of fats on the amount of DNOC absorbed from the gastrointestinal tract depends on the type and dose of the fat. In general, readily digested olive oil was associated with little change in DNOC blood levels, the more slowly digested rape seed oil slightly inhibited DNOC absorption, and castor oil decreased the absorption considerably. This latter result probably reflects castor oil's cathartic effect.

DNOC is rapidly absorbed by the skin in small quantities by humans (Batchelor et al. 1956; Harvey et al. 1951; Steer 1951) and rabbits (King and Harvey 1953a). Blood DNOC levels were increased by l–3 µg/g within <6 hours in three male volunteers who had an aqueous solution of DNOC dermally applied to the forearms (Harvey et al. 1951). In an experimental study, two volunteers initially placed one foot and subsequently both feet in a pail containing a 1% solution of DNOC (van Noort et al. 1960). Serum

DNOC levels were 2–4, 3–4, 7.5–8, and 27 µg/mL (roughly equivalent to µg/g) at 1, 2, 5.5, and 6.5 hours, respectively, after exposure. These data suggest that DNOC accumulated during the exposure period and probably very little was eliminated within this time. DNOC has also been detected in the blood of spray operators following occupational exposure of 63.2 mg/hour for 548 hours over 5 days (Batchelor et al. 1956). DNOC serum levels did not exceed 4.3 µg/g in six of these spray operators, and no correlation was apparent between total hours of exposure and serum levels. In a separate case study, 75 µg/g DNOC was recovered from the blood of a spray operator who died after dermal exposure to DNOC for an unspecified period (Steer 1951).

Blood DNOC levels peaked at 10–40 µg/g within l–2 hours in rabbits dermally exposed to 1 or 2 mg/cm^2 of DNOC (King and Harvey 1953a). A second dose of DNOC caused another increase in the blood DNOC values. Detection of blood DNOC 48 hours after exposure at levels higher with dermal exposure than for other routes suggests that skin acts as a reservoir for DNOC.

3.1.2 Distribution

Information regarding distribution of DNOC in humans and animals after inhalation exposure is limited. About 0.9 µg/g of DNOC was recovered from the cerebrospinal fluid of a worker exposed to unknown levels of DNOC during mixing and/or spraying operations over a 5-week period (Pollard and Filbee 1951).

Concentrations of 16, 20, 31, and 28 µg/g were recovered from the lungs of four rats exposed to 0.1 mg/m^3 DNOC for 4 hours (King and Harvey 1953a). The concentrations of DNOC in the alimentary tract and contents were 2.5, 3.1, 2.8, and 2.2 µg/g. The recovery of DNOC from the alimentary tract probably resulted from enterohepatic circulation and/or impaction of the aerosol along the trachea and bronchi and subsequent mucociliary action to bring it up to the epiglottis to be swallowed.

DNOC was detected in several organs of two humans who had committed suicide after ingesting unknown quantities of DNOC (Sovljanski et al. 1971). The following levels in each respective individual were: 13 and 400 mg/100 g in the stomach, 0.75 and 10 mg/100 g in the intestines, 0.3 and 4.72 mg/100 g in the liver, 0.125 and 2.0 mg/100 g in the kidneys, 0.3 and 2.42 mg/100 g in the heart, and 0.125 and 1.2 mg/100 g in the brain.

In two rats given oral doses of 0.4 mg/kg ^{14}C-DNOC, approximately 20–41% of the radioactive dose was distributed to other body tissues (Leegwater et al. 1982). In the rat killed 1 day after the dose, 15% of the dose was detected in the blood, 5.0% in the liver, 0.94% in the kidney, 0.08% in the spleen, 6.67% in the gastrointestinal tract, and 28% in the residual carcass. In the rat killed 3 days after dosing, 5.5% was detected in blood, 2.3% in liver, 0.9% in kidneys, 0.04% in spleen, 4.0% in gastrointestinal tract, and 12.6% in residual carcass.

No information was located regarding distribution of DNOC *per se* in animals after oral exposure, but the metabolite, 6-amino-4-nitro-*o*-cresol was detected in the liver, kidney, and brain of rabbits given single doses of DNOC (Truhaut and De Lavaur 1967). The ratio of 6-amino-4-nitro-*o*-cresol to DNOC increased from 0.42 to 5.29 in the kidney when the dose increased from 10 to 20 mg/kg DNOC.

DNOC was detected in unspecified tissues of a spray operator who died after dermal exposure to an unknown amount of DNOC (Steer 1951). About 0.9 µg/g of DNOC was recovered from the cerebrospinal fluid of a spray operator thought to have been exposed dermally and by inhalation to an unknown amount of DNOC over a 5-week period (Pollard and Filbee 1951). The blood level was approximately 37 µg/g on the same day, indicating a relatively smaller distribution to the cerebrospinal fluid.

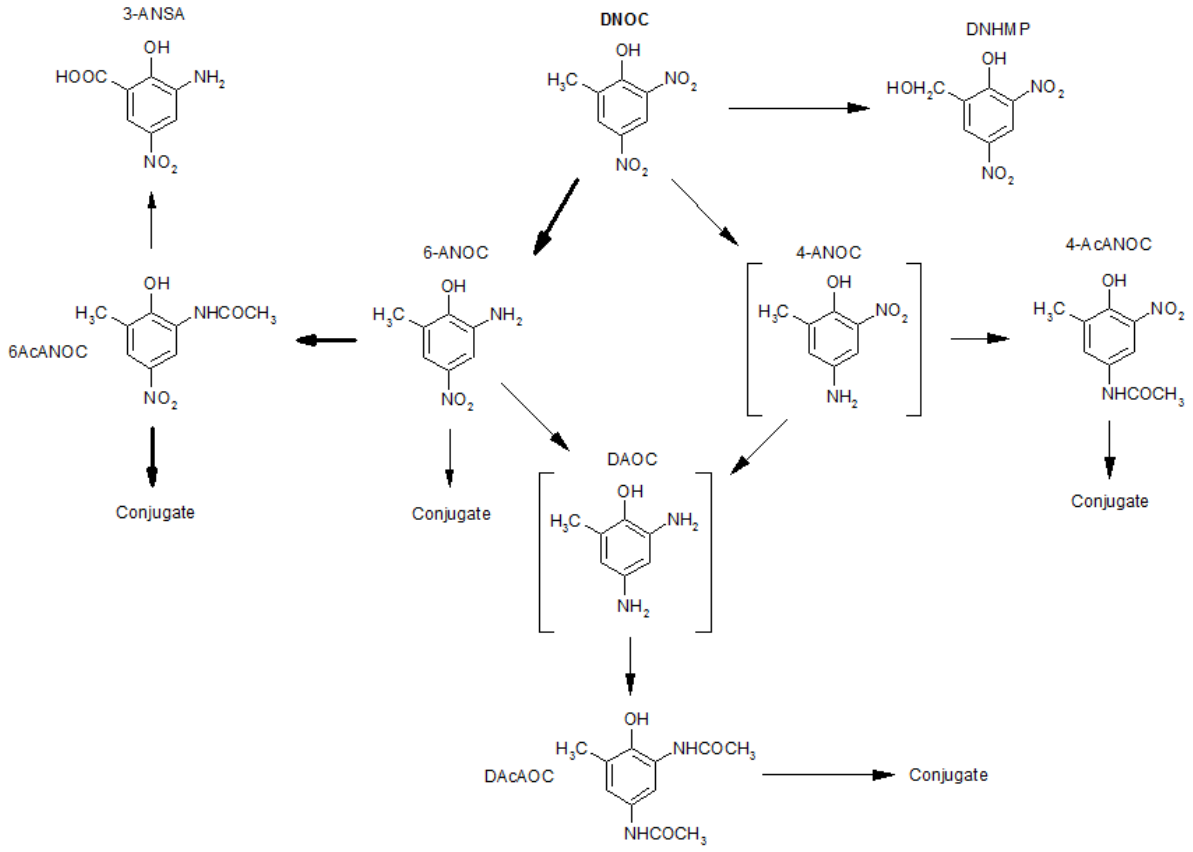
DNOC was measured in the serum, brain, spleen, kidney, liver, muscle, lung, and heart of rats at 30 minutes and 1, 2, 3, 4, 5, and 6 hours after a subcutaneous dose of 10 mg/kg (Parker et al. 1951). Except for liver and lung tissues, tissue DNOC increased from levels of 0.5–8.0 to 3.5–19 µg/g during the first 3 hours, but declined to levels of 1.5–10.5 µg/g during the next 3 hours. Liver levels fell from 14 µg/g at 30 minutes to 8 µg/g at 6 hours, while lung levels increased from 18 µg/g at 30 minutes to 20.5 µg/g at 2 hours and 30 µg/g at 6 hours. More DNOC was distributed to the lungs, heart, liver, and kidneys than to other tissues analyzed at the end of 6 hours. This can be attributed to increased blood supply to these organs and their relative affinity for DNOC. A single dose of 20 mg/kg DNOC resulted in DNOC tissue levels of 8, 7, and 45 µg/g in liver, kidney, and serum, respectively, 24 hours after the injection. A subcutaneous dose of 20 mg/kg/day for 40 days resulted in DNOC tissue levels of 7, 7, and 38 µg/g in liver, kidney, and serum, respectively, 24 hours after the last injection. The data suggest that there was no tendency for DNOC to accumulate in these body tissues. In addition, there was no difference in these tissue levels when the levels were compared 24 or 48 hours after the last injection (either single or multiple dose injections).

3.1.3 Metabolism

The metabolic fate of DNOC has been determined from a limited number of *in vivo* metabolic studies in experimental animals (Leegwater et al. 1982; Parker et al. 1951; Smith et al. 1953; Truhaut and De Lavaur 1967). In one study, no amino-nitrophenol, glucuronides, or ethereal sulfates were detected in urine from dogs or rabbits that received 10 mg/kg DNOC subcutaneously (Parker et al. 1951). Only DNOC was detected in the urine. The data from two other studies suggest that DNOC is biotransformed to less toxic metabolites in rats (Leegwater et al. 1982) and rabbits (Smith et al. 1953; Truhaut and De Lavaur 1967). Proposed metabolic pathways for 4,6-DNOC are presented in Figure 3-1. Unchanged DNOC and conjugated and unconjugated metabolites of DNOC were recovered from urine 2 days after rabbits received oral doses of 20–30 mg/kg DNOC (Smith et al. 1953). Less than 20% of the dose was excreted as metabolites; almost 5% of the dose was excreted as unchanged DNOC and 1% was excreted as conjugated DNOC. Therefore, the conjugation of DNOC represents a minor pathway. The metabolites were derivatives of 6-amino-4-nitro-*o*-cresol (6-ANOC; approximately11–12% of the dose). Approximately 1–1.5% of the dose was represented by 6-acetamido-4-nitro-*o*-cresol (6-AcANOC); approximately 10% of the dose was represented by O-conjugates of 6-AcANOC. Small amounts of 3-amino-5-nitrosalicylic acid (3-ANSA) and derivations of 4-amino-6-nitro-o-cresol (4-ANOC) were also excreted. The 6-nitro group appears to be more readily reduced than the 4-nitro group. According to the pathway, the acetylated metabolite (6-AcANOC) of 6-ANOC is further metabolized to traces of 3-ANSA and larger amounts of conjugates of 6-AcANOC. Metabolites 6-ANOC and 6-AcANOC were far less toxic than DNOC when oral doses were given to rabbits, suggesting that the major detoxification pathway in rabbits occurs via reduction at the 6-nitro group rather than via conjugation of the hydroxyl group of DNOC alone.

No amino derivatives of DNOC were detected in the blood, bone marrow, or adipose tissue, but 6-ANOC was detected in the liver, kidneys, and brain of rabbits that received an oral dose of 18 mg/kg DNOC (Truhaut and De Lavaur 1967). No 4-ANOC was detected in these tissues. Both DNOC and 6-ANOC were recovered from the urine as 25–38% of the dose. Smaller amounts of 4-ANOC were also detected in the urine. Further experiments demonstrated that as the dose of DNOC increased, the ratio of 6-ANOC to DNOC in urine increased. The data from this study support the findings of Smith et al. (1953) by demonstrating that the metabolic reduction of DNOC to 6-ANOC was the major detoxification pathway and that this pathway becomes more important at higher doses.

Figure 3-1. Proposed Metabolic Pathways for 4,6-Dinitro-*o*-Cresol (DNOC)



3-ANSA = 3-amino-5-nitrosalicylic acid; 4-AcANOC = 4-acetamido-6-nitro-*o*-cresol; 4-ANOC = 4-amino-6-nitro-*o*-cresol; 6-AcANOC = 6-acetamido-4-nitro-*o*-cresol; 6-ANOC = 6-amino-4-nitro-*o*-cresol; DAcAOC = 4,6-diacetamido-*o*-cresol; DAOC = 4,6-diamino-*o*-cresol; DNHMP = 4,6-dinitro-2-hydroxymethylphenol; DNOC = 4,6-dintro-*o*-cresol

Source: WHO 2000

The following urinary metabolites were identified and quantitated in a rat given 0.4 or 6.0 mg/kg ^{14}C-DNOC: 6-ANOC (l–2%); 6-AcANOC (2–3%); 4,6-dinitro-2-hydroxymethylphenol (DNHMP; 4–5%); 4,6-diacetamido-o-cresol (DAcAOC; 18%); and 4-acetamido-6-nitro-o-cresol (4-AcANOC; l–2%) (Leegwater et al. 1982). In addition, the urine contained several unknown metabolites and conjugates. In another experiment, the metabolites 6-ANOC, 6-AcANOC, and DAcAOC were also identified in a 24-hour urine sample from rabbits given 20 mg/kg. This study confirms findings of King and Harvey (1953a, 1953b), Smith et al. (1953), and Truhaut and De Lavaur (1967) showing slow elimination of

DNOC and reduction as the major metabolic pathway. The metabolites DNHMP and DAcAOC had not been previously found in rats.

Rat caecal contents were incubated with DNOC to determine whether the compound is metabolized in the large intestine (Ingebrigtsen and Froslie 1980). About 80% of DNOC was metabolized to 6-ANOC within 1 hour. Within the next 12 hours, 90% of this metabolite was further reduced to 4,6-diamino-*o*-cresol (DAOC). The authors determined that the caecal microorganisms in rats were responsible for the reduction of DNOC and its subsequent metabolites to diamino derivatives. Although not detected in humans or other monogastrics, these diamino derivatives are formed in sufficient quantities in ruminants to cause methemoglobinemia, which can be fatal in these species (Froslie 1973).

3.1.4 Excretion

Based on measured DNOC blood levels of a worker likely exposed to DNOC by a combination of inhalation and dermal routes (Pollard and Filbee 1951), an elimination rate constant of 0.002 $hour^{-1}$ and a half-life of 153.6 hours were determined (King and Harvey 1953b). A peak urinary quantity of 22 mg DNOC was found on the third day after the employee was admitted to the hospital and 5 weeks after his initial exposure (Pollard and Filbee 1951). About 89.9 mg of DNOC was eliminated via the urine over the 20 days after admission. The data suggest that humans have a relatively inefficient mechanism for eliminating DNOC and this may be due to slow detoxification and excretion or storage of DNOC in the body. In the only inhalation study located for animals, an elimination rate constant of 0.01 $hour^{-1}$ was determined for female hooded rats exposed to 2 mg/m^3 of DNOC aerosols for 5 hours (King and Harvey 1954). This was determined to correspond to an initial blood level of 60 µg/g that would result in essentially complete elimination of DNOC in 182 hours.

Urinary excretion data from five humans who each ingested 75 mg DNOC/day for 5 days suggested that at least 7% of the dose was slowly eliminated over a 13-day period (King and Harvey 1953b). The amount of DNOC excreted in the urine was independent of the concentration of DNOC in the blood of three humans. Species differences in urinary elimination have been found among animals administered DNOC orally. Elimination rate constants were 0.0105 and 0.0112 $hour^{-1}$ in rats given nine daily doses of 20 mg/kg/day DNOC and a single dose of 30 mg/kg DNOC, respectively (King and Harvey 1953b). The half-lives for DNOC were 26.8 and 28.5 hours for the multiple dose study and the single dose study, respectively. The authors calculated that an initial blood level of 60 µg/g DNOC will be eliminated almost completely from the blood within 182 hours. Higher elimination rate constants were obtained for

rabbits compared to rats; that is, 0.0448 $hours^{-1}$ for the multiple-dose study and 0.0454 $hours^{-1}$ for the single-dose study (King and Harvey 1953b). The half-lives were also shorter (6.6–6.7 hours) than those obtained for rats. Most of the excreted amount (average 6.4% of the dose) was eliminated through the urine in the first 5 hours. After comparing the data from humans, rats, and rabbits, the authors concluded that the rabbit is most efficient in detoxifying and eliminating DNOC. In another study, the elimination rate constants for DNOC in rats, rabbits, guinea pigs, mice, and monkeys given single unspecified oral doses of DNOC were 0.01, 0.045, 0.032, 0.036, and 0.01 $hours^{-1}$, respectively (Lawford et al. 1954).

In another oral rabbit study, DNOC and its metabolite, 6-ANOC, which were detected in the urine, made up 25–38% of a 10–15 mg/kg DNOC oral dose (Truhaut and De Lavaur 1967). Of this amount, 82–97% was eliminated within 1 day, and the rest was excreted within 2–3 days. As the dose of DNOC increased from 10 to 20 mg/kg, the ratio of 6-ANOC to DNOC in urine increased from 0.66 to 1.47 when measured at 2.5–3.75 hours postdosing.

Within 2 days after receiving a single oral dose of 20–30 mg/kg DNOC, Chinchilla rabbits excreted <20% of the dose as metabolites (Smith et al. 1953). Unchanged DNOC accounted for approximately 5% of the dose and conjugated DNOC accounted for approximately 1%. Derivatives of 6-ANOC comprised approximately 11–12% of the dose, including 6-AcANOC (1–1 .5% of the dose), O-conjugates of this metabolite (10% of the dose), and unspecified amounts of 3-ANSA and derivatives of 4-ANOC that were also excreted in the urine.

In two rats given oral doses of 0.4 mg/kg ^{14}C-DNOC, about 29–41% of the radioactive dose was excreted in urine and 10–23% was excreted in the feces (Leegwater et al. 1982). The half-life for elimination of radioactivity was 1–1.5 days. In the rat killed 1 day postdosing, 28.7% of the dose was excreted in the urine and 28% in feces. In the rat killed 3 days postdosing, excretion amounted to 23% of the dose in the first 24 hours, 16.4% in the next 24 hours, and 11.5% in the third 24 hours. Fecal excretion amounted to 7.1% in the first 24 hours, 9.3% in the second 24 hours, and 6.2% in the third 24-hour period. The following urinary metabolites were determined in a rat given 0.4 or 6.0 mg/kg ^{14}C-DNOC: DNOC (3–4%); 6-ANOC (l–2%); 6-AcANOC (2–3%); DNHMP (4–5%); DAcAOC (18%); and 4-AcANOC (l–2%). The urine contained several unknown metabolites and conjugates. The dose of DNOC had little effect on the distribution pattern of metabolites. In another experiment, the metabolites 6-ANOC, 6-AcANOC, and DAcAOC were identified in a 24-hour urine sample from rabbits given 20 mg/kg DNOC. This study confirms findings of King and Harvey (1953a, 1953b) and Smith et al. (1953), showing slow elimination of DNOC and reduction as the major metabolic pathway.

An average concentration of 0.8 µg/g DNOC with a range of 0.6–1.3 µg/g was detected in the urine from spray operators exposed dermally to 63.2 mg DNOC/hour (Batchelor et al. 1956). Of the 183 urine samples obtained from the spray workers, only 5 contained ≥0.5 µg/g DNOC as the sodium salt (limit of detection). Three of four spray operators, who were exposed to DNOC primarily by the dermal route for 14 days to 4 months, had initial serum DNOC levels of <5–100 µg/mL at the time of hospitalization (van Noort et al. 1960). In two of these patients, serum levels decreased from 60 to 40 µg/mL in 1 week and from 100 to 5 µg/mL in 3 weeks, respectively. Although the initial serum DNOC level was not determined in the fourth patient, 10 µg/mL DNOC was detected in the serum 1 month after exposure, suggesting that the initial serum level was extremely high. Thus, DNOC was eliminated slowly and at similar rates in these humans. A peak urinary DNOC excretion of 22 mg was observed on the third day after the employee was admitted to the hospital and 5 weeks after his initial combined dermal and inhalation exposure to DNOC (Pollard and Filbee 1951). A total of 89.9 mg of DNOC was eliminated via the urine over the 20 days after admission.

Urinary DNOC accounted for 10% of the total dose of 0.5–80 mg/animal over 3 days after the last, daily, subcutaneous injection of DNOC in rabbits and dogs (Parker et al. 1951). The determined elimination rate constants for DNOC were 0.02, 0.077, 0.021, 0.04, and 0.02 $hour^{-1}$ in rats, rabbits, guinea pigs, mice, and monkeys, respectively, following single intraperitoneal doses of DNOC (Lawford et al. 1954). Neither the sex of the test species nor the magnitude of the DNOC dose had a marked effect on the elimination of DNOC in rats given various intraperitoneal doses of DNOC (King and Harvey 1954). Elimination rate constants ranged from 0.013 to 0.019 $hours^{-1}$ for the 20 mg/kg dose and from 0.01 to 0.018 $hours^{-1}$ for the 5, 10, and 15 mg/kg dose groups. The determined mean elimination rate constant was 0.015 $hour^{-1}$ for this study.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use

mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

No PBPK models were located for dinitrocresols.

3.1.6 Animal-to-Human Extrapolations

Animal models may serve as indicators of potential DNOC-induced health effects relevant to humans. However, humans excrete DNOC and/or its metabolites much more slowly than do laboratory animals. An estimated elimination rate constant for internalized DNOC in humans was reported to be 0.002 $hour^{-1}$ (Pollard and Filbee 1951). Lawford et al. (1954) estimated elimination rate constants of 0.01, 0.045, 0.032, 0.036, and 0.01 $hours^{-1}$ for rats, rabbits, guinea pigs, mice, and monkeys given single oral doses of DNOC. King and Harvey (1953b) concluded that the rabbit is most efficient at detoxifying and eliminating DNOC. Evidence of slower elimination of DNOC from exposed humans and a lack of adequate comparative toxicokinetic data between humans and laboratory animals preclude meaningful interspecies extrapolations.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to dinitrocresols are discussed in Section 5.7, Populations with Potentially High Exposures.

No data identifying subpopulations of humans inherently more susceptible to the toxic effects of DNOC were located. Animal studies did not indicate that there were sex or age differences in the susceptibility to DNOC toxicosis.

Several human studies suggest that people living in tropical or warm climates are more susceptible to DNOC toxicity than people in cooler climates (Bidstrup and Payne 1951; Pollard and Filbee 1951; Stott 1951). This phenomenon is supported by studies in rats and mice indicating that environmental temperature increases the toxicity of DNOC (Harvey 1959; King and Harvey 1953a). Some human subpopulations that are predisposed to a syndrome known as malignant hyperthermia may be more likely to develop fatal hyperthermia following DNOC exposure. Malignant hyperthermia is an inherited disease of skeletal muscle characterized by a drug-induced hyperpyrexia (Schroeder and McPhee 1990). Human populations with this inherited disease are predisposed to acute hyperthermic reactions triggered by stress or drugs, such as anesthetic agents, skeletal muscle relaxants, and amide local anesthetics (Britt 1979). Although no data were located linking DNOC with malignant hyperthermia, persons with the genetic predisposition may be more susceptible to the hyperthermic effects of DNOC.

DNOC is an uncoupler of oxidative phosphorylation and causes metabolic disturbances. People with compromised metabolic rates may be more susceptible to DNOC toxicity; however, no such population has been identified.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to dinitrocresols are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for dinitrocresols from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by dinitrocresols are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Detection of DNOC in body fluids or tissues can serve as a qualitative indication that exposure to DNOC occurred. DNOC and/or its metabolites have been measured in various body fluids and tissues such as blood, urine, liver, stomach, intestine, brain, and heart of humans (Harvey et al. 1951; King and Harvey 1953b; Sovljanski et al. 1971) and animals (King and Harvey 1953a; Leegwater et al. 1982; Truhaut and De Lavaur 1967). Detectable blood and urinary levels of DNOC have been found in humans exposed occupationally by the inhalation and dermal routes (Batchelor et al. 1956; Bidstrup et al. 1952; Pollard and Filbee 1951; Steer 1951) or experimentally by the oral and dermal routes (Harvey et al. 1951; King and Harvey 1953b). Although the exposure concentrations in occupational studies were not known, the experiments in volunteers provide information on doses and durations. Thus, the measurement of DNOC in blood is a useful indicator of exposure; however, since DNOC is still detectable in the blood as much as 40 days after exposure, it may not be a reliable indicator of exposure history.

Following oral dosing of volunteers with DNOC, only 7% of the administered dose was excreted in the urine during 13 days postadministration (King and Harvey 1953b). Therefore, urinary levels of DNOC and/or its metabolites may not be useful biomarkers to quantify exposure. Yellow staining of skin, sclera,

or conjunctiva may alert a physician to the possibility of DNOC exposure. This yellow staining is not a sign of icterus, but is due to the yellow color of DNOC.

3.3.2 Biomarkers of Effect

DNOC exposure results in a hypermetabolic state that resembles heat exhaustion and heat stroke. The basal metabolic rate was increased by 70–100% within 3 days in two humans given 3 mg/kg/day DNOC (Dodds and Robertson 1933). Headaches, hyperthermia, profuse sweating, increased pulse rate, and dyspnea are other common signs and symptoms associated with DNOC exposure. In severe cases, tachycardia, delirium, coma, and convulsions are usually observed in humans. An increased basal metabolic rate may, therefore, indicate profound metabolic disturbances.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Very little information was located regarding interactions of DNOC with other chemicals, but the toxicity of DNOC is influenced by several physical and environmental factors.

Environmental temperatures influenced the mortality rate among rats after oral exposure to DNOC (King and Harvey 1953a). Six out of 12 rats died after being given 20 mg/kg at 37–40°C, while only 2 of 12 rats died after being given twice the dose (40 mg/kg) at almost half the temperature (20–22°C). It appears that increased environmental temperatures increased the toxicity of DNOC in rats. The authors further proposed that the increase in environmental temperature exacerbated the increased metabolic effect of DNOC, but did not appear to initiate or stimulate any reactions affecting the linkage of DNOC to any intracellular or extracellular substances. Environmental temperatures could also alter normal body functions so that the rate of absorption, diffusion, distribution, or metabolism of a compound would be changed. A similar observation was made in another study after rats given intraperitoneal doses of DNOC were exposed to 8, 26, or 36°C (Keplinger et al. 1959). The approximate lethal dose was 42 mg/kg at 8°C, 28 mg/kg at 26°C, and 18 mg/kg at 36°C. In mice given 22 mg/kg DNOC subcutaneously, the mean time of death (LT_{50}) values decreased as environmental temperature increased (Tesic et al. 1972). Hence, DNOC was most toxic at high temperatures and least toxic at cold temperatures.

An attempt was made to determine the best treatment regimen for rats and mice exposed to intraperitoneal doses of 2.5–30 mg/kg DNOC (Harvey 1959). Fifty percent mortality was observed at 2.5 mg/kg DNOC and 100% mortality was observed at ≥5.0 mg/kg when rats were exposed to 39–41°C. Sponging with

water within 1 hour of exposure to DNOC completely protected all rats given doses of 2.5–10 mg/kg. This protective effect was not observed at 20 or 30 mg/kg DNOC. Removal of the rats to a cold room completely protected the rats treated with 10 mg/kg, but had no effect on mortality at 20 mg/kg. The authors concluded that cooling of the skin may be beneficial in reducing the toxicity of DNOC in humans. Because rats eliminate DNOC more rapidly than humans, sponging and cooling treatment would have to be prolonged and efficient. In the same study, administration of 4-methyl-2-thiouracil, an inhibitor of the thyroid gland, 1 hour after injection of DNOC reduced mortality to 50% at 5.0 mg/kg, but had no effect on mortality at 10.0 mg/kg. No mechanism was proposed for this interaction between DNOC and 4-methyl-2-thiouracil. Similar results of sponging with water or treatment with 4-methyl-2-thiouracil were found with mice.

Mean time to death was prolonged in mice pretreated with vitamin E, vitamin A, and/or glucose 30 minutes before dosing with DNOC (Tesic et al. 1972). Thiamazole increased the mean time to death by a factor of 2.72, while chlorpromazine was more protective than thiamazole. The authors proposed that chlorpromazine may cause a significant reduction in oxidative processes and decrease in body temperature, while the protective effect of thiamazole may be associated with its ability to decrease basal metabolic rate.

Gavage administration of olive oil, rape oil, or castor oil to rats immediately after DNOC resulted in some alteration of blood DNOC levels, indicating that the influence of fats on the amount of DNOC absorbed from the gastrointestinal tract depends on the type and dose of the fat (Starek and Lepiarz 1974). In general, readily digested olive oil had little effect on blood levels, the more slowly digested rape oil slightly inhibited DNOC absorption, and castor oil decreased the absorption considerably. A nonpurgative dose of 0.2 mL of castor oil inhibited DNOC absorption from the alimentary tract, while a purgative dose of 1.0 mL inhibited absorption for the first 6 hours and then increased blood DNOC levels in the next few hours. In some instances, castor oil inhibited DNOC absorption by as much as 43–49% at 6 hours after the oil was given. Aspirin enhances uncoupling of oxidative phosphorylation and therefore increases DNOC toxicity (Ellenhorn and Barceloux 1988).